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Fluorescent influenza-like particles and control over their composition

Marc Aucoin
University of Waterloo, maucoin@uwaterloo.ca

FLUORESCENT INFLUENZA-LIKE PARTICLES AND CONTROL OVER THEIR COMPOSITION

Marc G. Aucoin, Department of Chemical Engineering, University of Waterloo, Ontario, Canada
maucoin@uwaterloo.ca

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Background

The Baculovirus Expression Vector System (BEVS) is a proven platform for making biologics. Despite being widely used, the system has typically relied on 'brute force' high expression. Building off of the handful of scientific publications that have shown that alternate promoter usage can promote higher functional protein yields, we have sought to generate baculovirus constructs that make use of a greater variety of promoters and genetic elements (shRNA/CRISPRi) to better control gene expression.

Materials and Methods

Genes for green fluorescent protein (GFP), DsRed2, and iRFP682, alone or as fusions to the human influenza A/PR/8/34 hemagglutinin (HA), matrix (M1) and neuraminidase (NA) proteins, respectively, were cloned into baculoviruses under the control of various insect cell promoters. Additional control elements (shRNA/CRSPRi), interfering with recombinant protein expression, were similarly constructed into the recombinant baculovirus. Insect cells (Sf9 and Hi5 cells) were synchronously infected with the single polycistronic baculoviruses and expression patterns were monitored. Cell fluorescence was monitored using an imaging flow cytometer. Virus-like particles were recovered from infected cell culture supernatants by subjecting the supernatants to iodixanol gradient ultracentrifugation. Virus-like particles were characterized by flow cytometry, by negative stain transmission electron microscopy (TEM), by multi-angle dynamic light scattering (MADLS) and Hemagglutination assay.

Results

Compared to the production of simple reporter proteins driven by various baculovirus promoters upon infection of insect cells, levels of fluorescence in cells infected with baculovirus carrying the fluorescent influenza protein fusion genes, under the control of the same promoter combinations, were very similar. This was true despite the fusion proteins having completely different localization in the cell. While the reporter proteins 'flooded' the cell to create cells with uniform fluorescence, the fusion proteins had distinct localization. The overall expression profiles have led to the extraction of mathematical parameters that can be used in the simulation of expression patterns and product formation.

Iodixanol gradient ultracentrifugation of the supernatants was able to isolate VLPs of approximately 70-85 nm having the distinct spike-like projections of influenza particles and that retained hemagglutination activity. Iodixanol purified VLPs subjected to analysis by flow cytometry revealed different fluorescent levels corresponding to the choice of baculovirus vector and promoter control used for the generation of the particles.

Conclusion

Our work shows that fine-tuned control is achievable in a system producing complex protein structures such as virus-like particles. Characterization of outcomes from the use of promoter control is leading to more robust and predictable protein expression. Finally, it has been shown that tailored protein expression can also modulate the composition of virus-like particles. Moving forwards, creation of virus-like particles with antigenic molecules from different origins but in set, and different, stoichiometries may be possible.